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By: Leslie Lindsay
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Date: January 2, 2003

Sir:

Enclosed for filing is a complete provisional patent application entitled "METHOD AND APPARATUS FOR MOLECULAR ANALYSIS IN SMALL SAMPLE VOLUMES" invented by:

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Specification including Claims – 23 pages
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Drawings - 3 sheets
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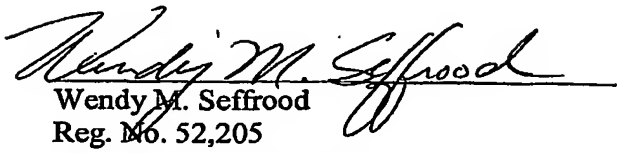

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METHOD AND APPARATUS FOR MOLECULAR ANALYSIS IN SMALL SAMPLE VOLUMES

BACKGROUND OF THE INVENTION

[0001] Understanding the nature of interactions between biomolecular and molecular species at both cellular and sub-cellular levels is key to the investigation of strategies for treating disease. One emerging methodology for elucidating the nature of molecular interactions involves the use of microarrays. Microarrays are spatially organized domains of various molecular species, and are typically constructed on solid supports arranged to facilitate rapid detection and characterization of molecular interaction events. Such events include interactions between biomolecules, antibodies and antigens, enzymes and substrates, and receptors and ligands, as well as biochemical and inorganic molecular events.

[0002] One benefit of microarray technology is the ability to provide a large number of test sites in a relatively small area. The size of the deposition domains, and in turn, the entire array, is of particular importance in determining the limits of sample volumes that can be tested.

[0003] There are four approaches for building conventional microarrays known in the art. These methods include mechanical deposition, *in situ* photochemical synthesis, "ink jet" printing and electronically driven deposition. Currently available mechanical deposition techniques produce domains of 25 to 100 microns in diameter or larger. *In situ* photochemical procedures allow for the construction of arrays of molecular species at spatial addresses in the 1-10 micron size range and larger. So-called "ink jet" methods produce domains in the 100 micron range. Electronic deposition can produce domains whose size is limited by the method used to construct the deposition electrode(s). Typically this is in the many

micron diameter size range. However, cellular and sub-cellular molecular events take place in volumes many times smaller than the above-described available domain sizes. Thus, what is needed is an apparatus and a method for interrogating extremely small sample volumes. This, in turn, would provide an opportunity for direct analyses of living cells *in vivo* or *in situ*.

[0004] Moreover, there remains a need for increased throughput and reduced costs associated with array production and utilization. More importantly, there remains a need for a method of analyzing extremely small sample volumes without requiring amplification of the material to be tested. A method of analyzing molecular events in living cells or tissue in near real time would also represent a substantial advance in the art. What is therefore described is a device and analytical platform for the evaluation of samples with volumes consistent with the contents of a single cell or smaller that provides for near real-time analysis, increased throughput and reduced costs.

SUMMARY OF THE INVENTION

[0005] The present invention includes an apparatus for analyzing a sample comprising a probe having a plurality of domains disposed thereon, wherein the domains form an array. Suitably, the array is a nanoarray. The domains suitably comprise biomolecules selected from the group consisting of drugs, chemical groups, lipids, DNA, RNA, proteins, peptide species, carbohydrates, and any combination of these entities. Optionally, nanosensors are operably connected to one or more of the domains.

[0006] The probe suitably comprises a microcantilever. In some embodiments, the probe is a dual element probe or a multielement probe. Some

embodiments of a probe of the invention comprise at least one microdisrupter disposed on the probe. Optionally, at least one microdisrupter comprises a tip or pointed member. The invention also encompasses probes comprising at least one hydrophobic region. Also described are embodiments wherein a suitable molecular detection device is operably connected to the probe. Suitable molecular detection devices include scanning tunneling microscopes, atomic force microscopes, mass spectrometers, fluorescence microscopes, flow cytometers, Raman spectrometers, Infra-red spectrometers, UV spectrometers, electronic systems, electrochemical systems, optical systems, magnetic and electromagnetic systems, and mass measuring systems.

[0007] Another aspect of the invention includes a method of detecting a molecular interaction event comprising the steps of contacting a sample with a probe having a plurality of domains disposed in an array, providing an incubation period, washing unbound molecules from the domains and detectin4g the molecular interaction event. Suitably, the sample comprises at least one cell or at least one cell lysate.

[0008] Also described is a method of detecting one or more molecules in a sample comprising the steps of contacting the sample with a probe having a plurality of domains disposed thereon, wherein the domains form an array, and wherein the domains are operably connected to one or more sensors, including nanosensors; and detecting binding of one or more molecules to one or more of the domains.

[0009] The present invention also provides a method of analyzing one or more analytes in a cell comprising the steps of disrupting a cell with a microdisrupter disposed on a probe, wherein the probe has a plurality of domains disposed thereon, and wherein the domains form a nanoarray; passing the nanoarray through the

membrane of the cell such that the nanoarray contacts intracellular space; and detecting the binding of one or more analytes to the nanoarray. Suitably, the method further comprises the step of passing the probe through the nuclear membrane such that the nanoarray contacts the intranuclear space. Alternatively, the method can comprise the step of inserting the probe into a cellular organelle. Cellular organelles suitable for analysis are those selected from the group consisting of a golgi complex, a mitochondria, a lysosome, an endoplasmic reticulum, a lipid raft, a cytoskeletal system, and any other physically or chemically definable cellular or sub-cellular domain or system.

[0010] The invention also encompasses a method of retrieving at least one analyte from a sample comprising steps of contacting the sample with a probe having a plurality of domains disposed thereon, wherein the domains form an array; and retrieving at least one analyte from the molecular domains.

[0011] Also provided is a method of delivering at least one substance to a cell comprising steps of reversibly attaching at least one substance to a probe having a plurality of domains disposed thereon, wherein the domains form an array; passing the probe through the membrane of the cell into the intracellular space; and releasing at least one substance into the intracellular space. Suitably, reversibly attaching at least one substance to a probe comprises contacting the substance to the domains such that a binding event occurs. Suitable substances include drugs, chemical groups, lipids, DNA, RNA, proteins, peptide species, carbohydrates and any combination of these entities. A suitable means of reversibly attaching comprises tethering at least one substance to at least one domain with a protease substrate. Additional methods include, but are not limited to, photolytic tethers, temperature sensitive tethers, ionically sensitive tethers, and chemically sensitive tethers.

BRIEF DESCRIPTION OF THE FIGURES

[0012] FIG. 1 is a schematic view of several embodiments of the invention showing mechanical micro4disrupter features.

[0013] FIG. 2 depicts use of an aqueous bridge with a probe of the invention having a microdisrupter and hydophilic and hydrophobic domains.

[0014] FIG. 3 depicts the use of the invention for direct interrogation of intracellular contents.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0015] The interrogation of extremely small sample volumes can be accomplished with the present invention. Provided are apparatuses including probes for analyzing a sample with an array. Suitable methods for using the probes of the invention are also provided.

Probes

[0016] As used herein, a "probe" refers to any suitable mechanical structure upon which an array can be composed and which can be used to interrogate a sample of small volume. Suitable probes include microfabricated structures. "Microfabricated structures" are millimeter, sub-millimeter or sub-micron scale structures and are generated by techniques known in the art including, but not limited to, laser ablation, electrodeposition, physical and chemical vapor deposition, photolithography, wet chemical and dry etching, injection molding, electron beam lithography, and X-ray lithography. Other suitable probe structures for use in the

present invention include biological microstructures such as eyelashes, cochlear hair cells, flagellum and actin filaments. Microcantilevers are also considered to be suitable for use as probes in the present invention and can include any of the above-described structures anchored at one or more ends or surfaces. Any portion of the cantilever can be used as a suitable anchor point. In some cases there may be multiple anchor points.

[0017] Optionally, a probe of the invention may include "microdisrupters," which, as used herein, are features that are suitable for disrupting a cell. Two mechanical embodiments of the microdisrupter feature are exemplified and depicted in Figure 1. "Disruption" of a cell includes any suitable technique by which the interior of a cell is accessed. "Disrupting" includes, but is not limited to, puncturing, penetrating, perturbing, oscillating, sonicating and lysing. Structures or features suitable for use as microdisrupters include tips or pointed members, serrated edges, pores, annulas, spheres or spherical members, enzymes such as lipases or proteases capable of digesting all or a part of a cell membrane, hypotonic or hypertonic compositions capable of altering the osmotic pressure of a cell and thermal or electromagnetic energy delivery devices including, but not limited to, photodiodes, lasers, electrical sources, temperature sources and radiowave sources. It should be noted that a probe having no additional microdisrupter disposed thereon may itself be used to access the interior of a cell through micromanipulation or the delivery of energy, enzymes or compositions as described above.

[0018] Probes of the invention are not limited to single element structures. For example, dual or multi-pronged probes are included within the scope of the invention. Each element, or "tine," of a multi-pronged probe can include an array. Arrays on adjacent prongs can be identical or can be different, having domains of

different species, or even different types of molecules. For example, one prong can have an array of DNA species and an adjacent prong can have an array of peptide species. Some prongs may not have an array disposed thereon. Additional prongs, if present, may serve the further function of disruption as described above, and may also include microdisrupters disposed thereon.

[0019] Probes of the invention may include anti-wicking features to prevent capillary action from drawing the sample away from the array. Suitable features include hydrophobic domains and mechanical structures that are physical barriers to wicking. Hydrophobic domains may be disposed on the surface of the probe or may be an integral component of the probe. Hydrophobic domains may comprise any portion of the probe, but are suitably constructed so as to facilitate maintained contact between the sample and the array. In this regard, hydrophobic domains may be used in conjunction with hydrophilic domains, which are most suitably disposed adjacent to, or as a substrate for, the array. Mechanical structures that are suitable for use in preventing wicking include O-ring structures, micro-dikes, micro-walls, bumps, protrusions, holes, cavities, filters and temperature gradients.

Arrays

[0020] As used herein an "array" refers to a plurality of spatially arranged domains disposed in known locations, or "addresses" on a probe of the invention. A "nanoarray" is an array in which each domain has a total area of less than about one micron. A "domain" or a "molecular domain" or an "affinity domain" is a discrete region of immobilized species including, but not limited to, chemical species, biomolecular species such as nucleic acids and peptides, and molecular and sub-molecular species. Specific non-limiting examples include antibodies, DNA, RNA,

normally or abnormally expressed cellular proteins, pathogens and antigens derived therefrom, reactive organic and inorganic chemical groups and multi-component complexes. It should be noted that as used herein, "peptide species" can include single amino acids, peptides, polypeptides and/or proteins.

[0021] Domains may further include nanosensors coupled to the immobilized species. "Nanosensor," as the term is used herein, refers to any reporter system that enables direct detection of interaction events or molecular activities occuring on the micron or smaller scale. The construction of suitable nanosensors for use in the present invention are described in copending application serial number 09/974,755, entitled "Nanoscale Sensor" which is incorporated herein by reference in its entirety. Briefly, nanosensors provide for the monitoring of nanoscale events by the detection of measurable changes in physical position, mass, electrical capacitance, conductivity or resistance, resonance frequency, resonance amplitude, morphology, kinetic energy, local temperature, oxidation/reduction (redox) state, structural integrity, bonding energy or other properties of the array species. Suitable structures for use as nanoscale sensors include carbon nanotubes, fullerene structures, nanobars and nanowires.

[0022] Arrays can be constructed on probes by any suitable methodology. One technique used in the construction of ultraminiturized arrays suitable for use in the present invention is described in copending application serial number 09/929,865, entitled "Nanoscale Molecular Arrayer," incorporated herein by reference in its entirety. This technique operates via piezoelectric, mechanical, magnetic or other methods for manipulation of a probe to deposit and reproduce domains smaller than about 1 micrometer to as little as ten nanometers or less. Briefly, a suitable method for constructing arrays includes steps of loading deposition materials on a deposition

probe and transferring the materials to a deposition substrate using an apparatus having X, Y and Z controllers for manipulation of the probe, a humidity controller, and a control computer. Additional optional components of an apparatus suitable for constructing arrays include a force feedback monitor and an optical microscope.

[0023] The ultraminiaturized attributes of some probes of the invention allow the construction of arrays with dimensions on the scale of a few microns and with molecular arrays formed from at least 2 to about 250 molecular domains of smaller than 1 micrometer down to as little as 10 nanometers or less each.

Molecular Detection Devices

[0024] As used herein, "molecular detection devices" include devices suitable for reporting microscopic or submicroscopic events on a macroscopic scale. The ability to measure events that occur on minute scales and report these events in the macroscopic world is of clear utility. One device suitable for the direct detection of molecular interaction events occurring at the micro- or nano-scale level is the scanning probe microscope. One type of scanning probe microscope is the atomic force microscope ("AFM"). In atomic force microscopy, the interactions between a sharp, micron-scale probe and a sample are monitored and regulated as the probe raster scans over the sample. Extremely fine control of the motion of the AFM probe is achieved using piezoelectric crystals. The AFM is capable of about two nanometer (or less) lateral resolution and less than one Angstrom vertical resolution. It can be operated in a vacuum, in atmospheres of varying humidity or in physiological solution, and is capable of identifying and measuring molecular binding events in near-real time. The resolution of the AFM can be very high, even on the atomic scale in some cases.

[0025] In addition to its high spatial resolution, the AFM is capable of exerting and detecting forces in the picoNewton (pN) range. This is the force range relevant to the forces extant between and within molecules. Thus, the AFM can measure intermolecular, as well as intramolecular bonding, or "rupture," forces. This is accomplished by repeated cycling of the AFM probe through an approach/retract sequence. Moreover, the AFM can measure a wide variety of other forces and phenomena, such as magnetic fields, thermal gradients and viscoelasticity.

[0026] Ultraminiaturization of molecular arrays is the next step in the evolution of microarray methodologies. Through ultraminiaturization, vast increases in throughput can be achieved, along with reductions in costs. Moreover, ultraminiaturization allows for the utilization of such small sample volumes that the methods necessary for recovery of sample materials can be virtually non-invasive, thereby greatly enhancing the comfort level of the sample donor. For example, rather than a painful tissue biopsy, a few cells obtained by a simple swab technique can provide the same level of information. Ultraminiaturization of arrays would allow for *in situ*, and even *in vivo*, detection of molecular and biomolecular events in real time, without the need for sample retrieval. Nonetheless, to date, no viable methodologies or devices for accomplishing these goals have been described.

[0027] Microscopic or submicroscopic events include intermolecular and intramolecular interaction events. One measurable intramolecular event is known as a "rupture event," and is defined herein as the force necessary to induce the breaking of intramolecular bonds. Other typical events that are suitably measured and reported by molecular detection devices include the binding of one molecular species to another molecular species via covalent, non-covalent, hydrophobic, electrostatic or hydrogen bonding, or a combination of these or other bonding mechanisms. Non-limiting

examples useful in the investigation of disease and therapeutic strategies include antibody-antigen interactions, receptor-ligand interactions and enzyme-substrate interactions.

[0028] Methods of molecular detection suitable for use in the present invention include inverse cyclic voltametry and other methods using electronic platforms, including but not limited to piezoelectric, capacitance, electromagnetic and laser-based devices. Other methods include the use of chemical reactions, changes in mass, bonding force, redox state, structural integrity, fluorescence, absorbance, quenching, local structural variation, kinetic energy, thermal energy, magnetic or electromagnetic reactivity, radio energy generation or absorption, general energy state and radioactivity to report binding events.

[0029] As discussed, the atomic force microscope is one instrument that is particularly useful in practicing an embodiment of the present invention. Other suitable instruments include scanning tunneling microscopes, mass spectrometers, fluorescence microscopes, flow cytometers, Raman spectrometers, Infra-red spectrometers, UV spectrometers, electronic systems, electrochemical systems, optical systems, magnetic and electromagnetic systems, and mass measuring systems. As discussed above, nanosensors can also be used to report molecular events. Coupling nanosensors to an electronic measuring device including, but not limited to an amp meter, conductivity meter, ohm-meter, or oscilloscope allows for the macroscopic detection of binding and other molecular events.

[0030] Molecular detection devices can be operably connected to probes of the invention. As used herein, "operably connected" refers to electric, magnetic, mechanical, optical, pneumatic or other means of connecting the probe and the

molecular detection device such that the macroscopic reporting of the molecular interaction event can be made simultaneously or in near-real time.

Methods

[0031] Probes of the invention may be used *in situ* or *ex situ*. As used herein, "*in situ*" usage refers to direct detection or measurement of molecular or sub-molecular events upon introduction of the array at the site of interest. For example, *in situ* usage includes *in vivo* interrogation of a sample with a probe. In contrast, "*ex situ*" usage refers to removing the sample from the site of interest prior to interrogation with the probe.

[0032] A probe of the invention can be used to directly interrogate a single living or non-living cell, as shown in Figure 3. Methods of isolating single living cells are known in the art. For example, U.S. Patent No. 6,420,105, incorporated herein by reference, describes a method of isolating and harvesting a single cell from its organ tissue using a device capable of collecting cells so that they remain substantially intact. Positioning and motion of the probe is accomplished using piezoelectric or similar motion control devices. In some embodiments, it is possible to specifically target subcellular domains such as the nucleus, or a specific organelle, such as a Golgi body. Suitably, a probe having a pointed member or other microdisrupter device situated thereon is inserted directly into a cell or positioned adjacent to the cell. Alternatively, a probe without a microdisrupter device can enter a cell or the cell can be lysed by any suitable means prior to interrogation. The components of the cellular environment are then allowed to interact with the molecular array on the probe.

[0033] In some cases, the amount of applied vertical force exerted by the probe on the sample is regulated by monitoring the degree of flexion of the probe using such methods including but not limited to strain gauges, optical lever systems, integrated piezo resistive methods, or other suitable methods. Motion of the probe can be in the X,Y plane and in the Z plane. In addition, ultrasonic energy can be imparted by rapid oscillations of the probe. These motions are accomplished using piezo ceramic motion control mechanisms, mechanical methods or other methods that are known to skilled practitioners in the art.

[0034] The array can contact the sample by any suitable direct or indirect means. An example of an indirect means of contacting a sample includes the use of an aqueous bridge, as shown and depicted in Figure 2. Hydrophilic and hydrophobic domains on the probe can be advantageously used to maintain contact between the sample and the array. When an aqueous bridge is used, a small drop of fluid deposited on the sample cell is captured between the probe and the cell and supporting substrate as shown in Figure 2. The sample is mechanically disrupted by motion of the probe and contact with the microdisrupter. As the sample is disrupted, the materials released diffuse through the aqueous bridge and contact the molecular domains on the probe. Specific capture agents on the array bind to components of interest contained in the sample. As discussed above, binding events are monitored by a variety of methods including, but not limited to, atomic force microscopy, fluorescence, Raman and IR scattering, mass spectrometry, electronic signatures, or changes in mechanical or resonance properties of the probe itself.

[0035] Biomarkers are one type of suitable target molecule for probes of the invention. As used herein, a "biomarker" is any molecule that can be used as an identifier of a particular cell, cell type, cell state, physiological state of an organ,

organ system, or whole organism, tissue, tissue type, tissue state, predisposition to disease including but not limited to cancer, drug tolerance, cytotoxicity effects and mental or psychological function. Typically, biomarkers are proteins, but can also be cell-surface peptides, intracellular peptides, lipids, carbohydrate moieties, RNA transcripts and/or DNA molecules, chemical groups, and/or circulating antigens.

[0036] Another suitable target for molecular analysis using methods of the invention is body fluid. A "body fluid" may be any liquid substance extracted, excreted, or secreted from an organism or tissue of an organism. Body fluid may or may not contain cells. Body fluids of relevance to the present invention include, but are not limited to, whole blood, plasma, serum, urine, cerebral spinal fluid, tears, sinovial fluid, semen, mucus and amniotic fluid.

[0037] Probes of the invention can also be used to retrieve an analyte from a complex solution. As used herein, an "analyte" refers to any substance, molecule of interest, biomolecule of interest, or particle for which information is desired. In this aspect, the arrays of the invention suitably comprise molecular domains capable of reversibly binding the analyte either for direct measurement on the probe, or for release and subsequent measurement by further analytical techniques. As will be appreciated by those of skill in the art, this embodiment can also be used to concentrate an analyte in a complex solution.

[0038] A further embodiment of the present invention is a method of delivering one or more substances to a living or non-living cell, tissue, or organism. In this embodiment, the domains of an array are "loaded" with the substance or substances to be delivered, which is suitably attached to the molecular domains by, for example, a protease labile tethering molecule. Suitable protease labile tethering molecules comprise a peptide sequence that is susceptible to being hydrolyzed by one

or more proteases found in the target cell, tissue or organism. Some non-limiting examples of protease labile tethering molecules include short-chain peptide substrates of serine proteases, metalloproteases, aspartate proteases and cysteine proteases. Additional tethering molecules include, but are not limited to, ion sensitive tethers (e.g., leucine zippers, chelaters (EDTA)), temperature sensitive tethers (e.g., PNA, DNA or RNA), photosensitive tethers, or chemically sensitive tethers. Substances that are suitable for delivery by probes of the invention include genes, polynucleotides comprising coding sequences, enzymes involved in DNA replication, transcription or translation, enzymes involved in cellular metabolism or other processes, restriction endonucleases, ligases, reactive species such as free radicals, drug candidates and drugs. As will be appreciated, the molecular domains of a probe can be loaded for delivery of multiple molecules of the same substance or different substances, which may or may not act in concert. For example, a gene of interest can be delivered to a living cell simultaneously with enzymes that can be used to splice it into the appropriate site of the host DNA.

[0039] Additional details of the invention will become more apparent by reference to the following non-limiting examples.

EXAMPLES

Prenatal and Neonatal screening

[0040] A small amount of prenatal (e.g., amniotic) or neonatal material is obtained. This material may be a blood sample, serum sample, body fluid, cell sample or any other biological sample for which a genetic or biomarker screen is desired. In the case of blood, a microdrop of the material is prepared by pipetting onto a glass slide that is maintained in a humid environment to prevent evaporation.

A nanoarray probe is brought into close proximity to the microdrop and inserted into the drop to allow the biomaterials on the drop to contact the molecular domains on the chip on a tip. After a suitable incubation period, the probe is removed from the microdrop, and the array is washed and analyzed by fluorescence, atomic force microscopy, or other methods known to those practicing the art.

[0041] In an alternative embodiment of this example, a small number of cells are obtained form a patient and maintained in a living state on a suitable substrate such as a glass slide or silicon chip. A nanoarray probe is carefully thrust through the cell membrane and allowed to interact with materials within the cell's cytoplasm, nucleoplasm or other sub-cellular location. After a suitable incubation period, the probe is removed from the cell, rinsed and evaluated as described above.

Forensics

[0042] Typical forensic samples include cellular materials, body fluids and trace chemicals. In one application of the present invention, a blood sample is recovered from a crime scene. There is insufficient material to complete a protein-based biomarker screen or a DNA fingerprint analysis without amplification. In one embodiment, a protein biomarker array on a probe of the invention is brought into proximity with the sample which has been resuspended in a minimal volume (less than one microliter) to maintain the highest concentration possible of low copy number protein biomarkers. After a suitable incubation period, the probe is processed as described above and a protein biomarker profile is obtained and can be used as a "signature" to identify or rule out a suspect.

Minimally invasive cancer diagnostics

[0043] In many cases, the acquisition of necessary biopsy material for a diagnostic cancer screen is a very painful process for the patient. This is largely due to the relatively large amount of biopsy material necessary for adequate testing. Use of an ultraminiturized array on a microprobe greatly decreases the amount of required material for a diagnostic screen, opening the door to methodologies that enhance patient comfort considerably. For example, rather than a major surgical procedure to obtain a suspect breast tumor, a relatively small needle is inserted into a tumor with minimal discomfort, and a small number of suspect cells is withdrawn. A cancer biomarker specific probe is juxtaposed to the cells and either the insertion or disruption technique is used to analyze the cellular content for cancer specific biomarkers.

[0044] In an alternative embodiment, rather than a painful surgical procedure to remove a suspect throat tumor, a much less painful throat swab is carried out to obtain just a few of suspect cells for analysis. The cells are analyzed as described above, again substantially improving levels of patient comfort over currently available methods of analysis.

Release of biomaterials into cells

[0045] In this example, rather using the probe to recover materials, a reverse procedure is carried out. A probe is "loaded" with a variety of materials, for example, DNA splicing enzymes, that are bound to specific sites on the array. The probe is then inserted into a specific cell or group of cells. By using a protease labile tether method, the biomaterials are released within the cells and allowed to carry out their bioactivity in a very cell specific fashion. This multiplexed delivery of materials to

specific cells provides for the retention of materials in an unreacted, "dormant" state on the probe until they are inserted into the cells and allowed to mix. This is applicable in scenarios where very site-specific modification of cells is desirable, such as in gene therapy embodiments.

Transgenic analysis

[0046] In this example, the goal is to evaluate small numbers of cells for their ability to grow into healthy transgenic animals. It is known that at early divisional stages of embryogenesis, it is possible to remove single cells without disrupting the growth of the embryo, assuming the embryo is otherwise normal and healthy. However, embryos that are morphologically normal can carry aberrant genes or metabolic anomalies that will result in unhealthy or dead newborns. To avoid this, it is desirable to carry out a biomarker profile of the embryo at an early stage. In this scenario, the probe is diagnostic for a group of biomarkers that are indicative of normal cellular growth and function. A single cell is removed from the embryo at an early stage. The probe is inserted into or used to disrupt the cell and the cell contents allowed to interact with the affinity domains on the probe. The probe is subsequently processed and the biomarker screen used to make a determination as to the health and utility of the embryo long before the expense and technical difficulty of carrying a defective transgenic animal to term are encountered.

Complex biopsy screening

[0047] A popular method for isolating different cell types from complex tissues is known as Laser Cell Microdissection ("LCM"). In this method, a laser is used to cause adherence of specific cells to an adhesive backing which is then

removed with the cells intact. These cells can be processed by conventional PCR methods to amplify DNA content, but the cell number is typically far too low to enable processing of protein profiles. A probe of the invention carrying the desired protein profiling affinity agents on the array can be used, either by insertion or disruption, to analyze the protein content of these dissected cells.

[0048] It should be noted that, as used in this specification and the appended claims, the singular forms "a," "an," "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to a method of detecting "a biological event" includes a method of detecting multiple biological events. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.

[0049] All publications and patent applications in this specification are indicative of the level of ordinary skill in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated by reference.

[0050] The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

We claim:

1. An apparatus for analyzing a sample comprising a probe having a plurality of domains disposed thereon, wherein the domains form an array.

2. The apparatus of claim 1, wherein the array is a nanoarray.

3. The apparatus of claim 1, wherein the domains comprise one or more biomolecules selected from the group consisting of drugs, drug candidates, chemical groups, lipids, DNA, RNA, proteins, peptide species, carbohydrates, and any combination thereof.

4. The apparatus of claim 1, further comprising nanosensors operably connected to one or more of the domains.

5. The apparatus of claim 1, wherein the probe comprises a microcantilever.

6. The apparatus of claim 1, wherein the probe is a dual element probe.

7. The apparatus of claim 1, wherein the probe is a multielement probe.

8. The apparatus of claim 1, wherein the sample comprises a volume of about 50 femtoliters to about 10 microliters.

9. The apparatus of claim 1, further comprising at least one microdisrupter disposed on the probe.

10. The apparatus of claim 9, wherein at least one microdisrupter comprises a tip or pointed member.

11. The apparatus of claim 1, wherein the probe further comprises at least one hydrophobic region.

12. The apparatus of claim 1, further comprising a molecular detection device operably connected to the probe.

13. The apparatus of claim 12, wherein the molecular detection device is a scanning tunneling microscope, atomic force microscope, mass spectrometer, fluorescence microscope, flow cytometer, Raman spectrometer, Infra-red spectrometer, UV spectrometer, electronic system, electrochemical system, optical system, magnetic and electromagnetic system, or mass measuring system.

14. A method of detecting a molecular interaction event comprising the steps of:
contacting a sample with a probe having a plurality of domains disposed in an array;
providing an incubation period;
washing unbound molecules from the domains; and
detecting the molecular interaction event.

15. The method of claim 14 wherein the sample comprises at least one cell.

16. The method of claim 14 wherein the sample comprises at least one cell lysate.

17. A method of detecting one or more molecules in a sample comprising:
contacting the sample a probe having a plurality of domains disposed thereon, wherein the domains form an array, and wherein the domains are operably connected to one or more nanosensors; and
detecting binding of one or more molecules to one or more of the domains.

18. A method of analyzing one or more analytes in a cell comprising the steps of:
disrupting a cell with a microdisrupter disposed on a probe, wherein the probe has a plurality of domains disposed thereon, and wherein the domains form a nanoarray;
passing the nanoarray through the membrane of the cell such that the nanoarray contacts intracellular space; and
detecting the binding of one or more analytes to the nanoarray.

19. The method of claim 18, further comprising the step of passing the probe through the nuclear membrane such that the nanoarray contacts intranuclear space.

20. The method of claim 18, further comprising the step of inserting the probe into a sub-cellular species.

21. The method of claim 20 wherein the sub-cellular species is selected from the group consisting of a golgi complex, a mitochondria, a lysosome, an endoplasmic reticulum, a lipid raft and a cytoskeletal system.

22. A method of retrieving at least one analyte from a sample comprising:
contacting the sample with a probe having a plurality of domains disposed thereon, wherein the domains form an array; and
retrieving at least one analyte from the molecular domains.

23. A method of delivering at least one substance to a cell comprising:
reversibly attaching at least one substance to a probe having a plurality of domains disposed thereon, wherein the domains form an array;
passing the probe through the membrane of the cell into the intracellular space; and
releasing at least one substance into the intracellular space.

24. The method of claim 23 wherein reversibly attaching at least one substance to a probe comprises contacting the substance to the domains such that a binding event occurs.

25. The method of claim 23 wherein at least one substance is DNA, RNA, a peptide species, a chemical, a drug or a reactive species.

26. The method of claim 23 wherein reversibly attaching comprises tethering at least one substance to at least one domain with a protease substrate, a photolyzable tether, a chemically reactive tether, an ionically reactive tether or a thermally sensitive tether.

27. A method of detecting an *in situ* molecular interaction event comprising the steps of:

contacting a sample with a probe having a plurality of domains disposed in an array;

providing an incubation period; and

detecting the molecular interaction event.

ABSTRACT

The interrogation of extremely small sample volumes can be accomplished with the present invention. Provided are probes having disposed thereon a plurality of domains forming an array, which is suitably a nanoarray. Also provided are methods of detecting molecules and molecular interaction events, retrieving and analyzing analytes, and delivering substances to cells or tissues using probes of the invention.

Figure 1

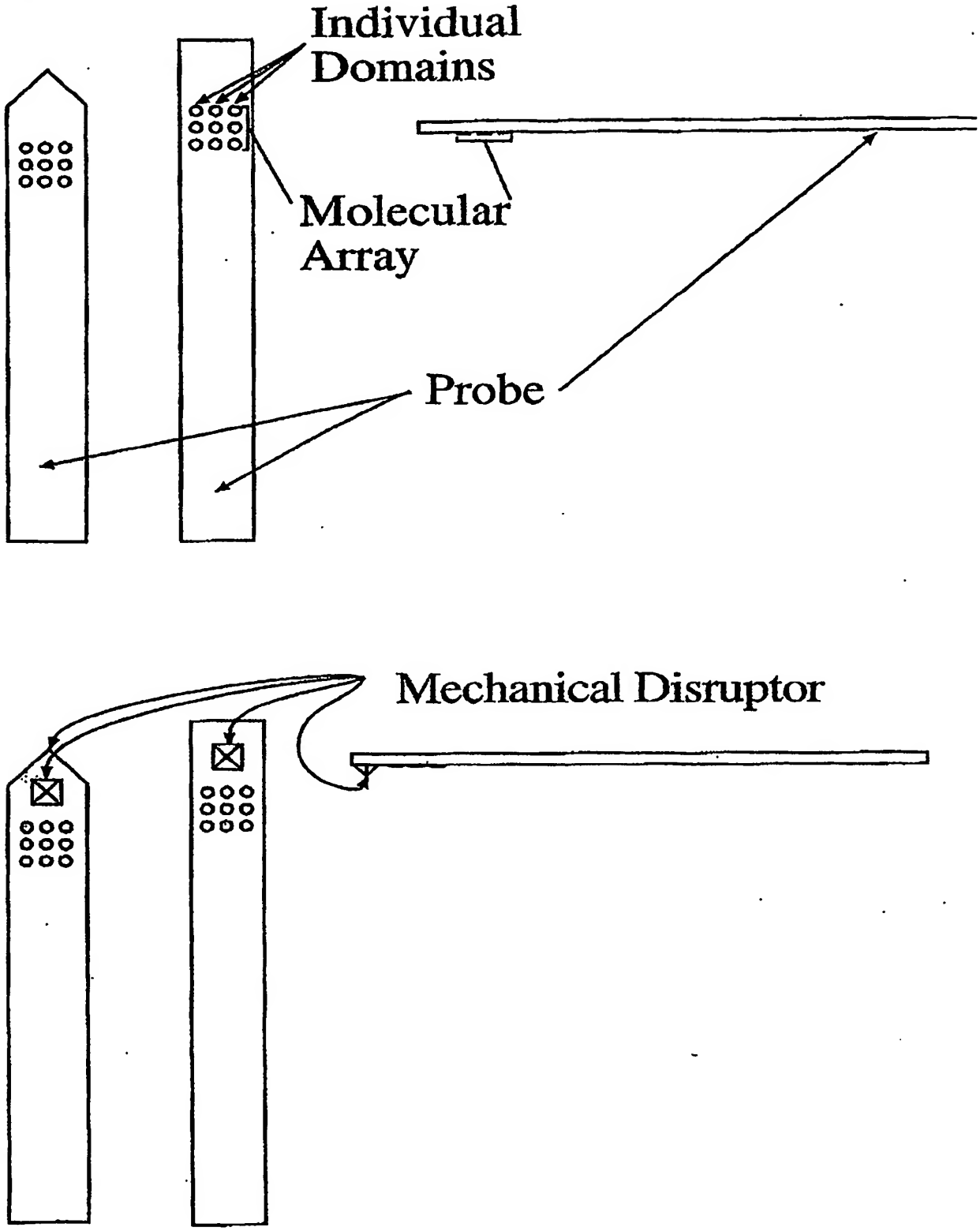
Individual
Domains
Molecular
Array
Probe
Mechanical Disruptor

Figure 2

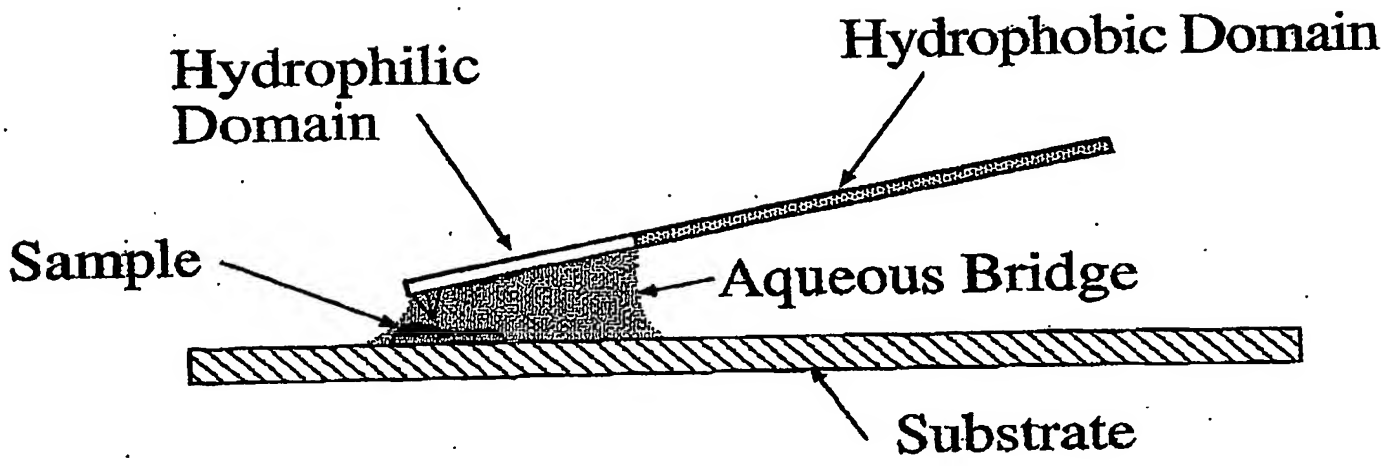
Hydrophilic Domain
Hydrophobic Domain
Sample
Aqueous Bridge
Substrate

Figure 3